Influence of Free Fatty Acids on Sweet Cream Butter Flavor

SUMMARY—A threshold pattern for the even-numbered free fatty acids (FFA) in butter depended on chain-length. Butyric acid had the lowest total average flavor threshold (AFT) of the more volatile FFA, and the total AFT values increased as chain-length increased through hexanoic and octanoic acids. A soapy after-taste predominated at and above the AFT for decanoic and dodecanoic acids. Threshold values decreased from octanoic acid through dodecanoic acid as the chain-length increased. The determination of AFT values for FFA in butter allowed an estimation of the importance of fatty acids in butter flavor. Mixture threshold results obtained support the concept that flavor components interact at subthreshold concentrations. Decrease in preference was shown for butter containing suprathreshold levels of total FFA.

INTRODUCTION

ENDOGENOUS levels of free fatty acids (FFA) are believed to contribute to the flavor of sweet cream butter. Excessive concentrations of volatile short-chain FFA are recognized as the cause of hydrolytic rancidity flavor in dairy products. The FFA content of several sweet cream butteroils has been reported by Bills et al. (1963) and Iyer et al. (1967). Their results showed considerable variations between samples. Scanlan et al. (1965) found that even-numbered fatty acids from butyric to dodecanoic accounted somewhat equally for the fatty acid contribution to rancid milk flavor.

FFA thresholds for butyric through dodecanoic have been determined in water (Patton et al., 1964; Siek et al., 1968) and for butyric through lauric in edible oil (Feron et al., 1961). The threshold for added butyric acid has been determined in deodorized butteroil (Siek et al., 1968) and added butyric and hexanoic acid thresholds have been determined in milk (Patton, 1964).

The purpose of this investigation was to find the thresholds for the C_4-C_{12} FFA in fresh sweet cream butter. This was accomplished by determining the amount of each individual FFA to add to butter in order to reach its added threshold value. This added value was combined with the endogenous level of each acid in the butter to give a total threshold value.

A second objective was to demonstrate the synergistic flavor interaction for mixtures of FFA in butter. Day et al. (1963) previously have discussed additive interactions of other flavor compounds at subthreshold concentrations.

Finally, the flavor preference for butters containing added FFA was determined.

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EXPERIMENTAL

Fatty acids

Reagent grade (Eastman Distillation Products) butyric, hexanoic, octanoic, decanoic and dodecanoic acids were used. Butyric, hexanoic and octanoic acids were vacuum distilled in a glass distillation apparatus (Siek et al., 1968). Decanoic and dodecanoic acids were purified by three consecutive ethanol-water recrystallizations. All acids were examined by thin-layer and/or gas chromatography, and were found to be at least 99.5% pure.

Sweet cream butter

High quality fresh sweet cream secured from commercial sources was pasteurized and churned in the University Dairy Products Laboratory, and was immediately frozen and held at −23°C until used. The storage time interval never exceeded 28 days.

Three lots of butter were used throughout the flavor panel testing: Lot I for individual FFA thresholds, Lot II for interaction thresholds and Lot III for preference evaluations.

FFA analysis

The procedure followed for FFA analysis was that of Bills et al. (1963), an adaptation of the original method reported by Hornstein et al. (1960), except that FFA were isolated from intact butter instead of isolated milk fat.

Preparation of butter test samples

Sweet cream butter was allowed to warm at ambient temperature to a workable state (ca. 16°C). Measured amounts of FFA were added at levels to include concentrations below and above the anticipated flavor threshold. After a thorough mixing by kneading in a plastic bag, the butter was formed into long-pound units and stored at 10°C for 24 hr prior to testing. Prior to serving, the butter was cut into patties and placed on coded paper butter chip plates. The serving temperature of the butter was approximately 16°C.

Threshold panel selection

A series of 21 triangle tests, using varying levels of added butyric acid in sweet cream butter, was presented to 25 people. From these tests 14 judges were chosen for their ability to detect butyric acid in butter. Judges selected for the panel scored correct responses on 50% or more of the triangle tests. The judges were experienced panel members but were not trained butter judges. Each judge was trained to detect individual fatty acid flavors when each acid was added at a high level to sweet cream butter.

Conflicts prevented three judges from participating throughout the entire testing period. New judges were evaluated by triangle tests as mentioned above, and were added to the panel as needed.

Individual compound threshold panel tests

Tests were conducted at 10:00 am and 3:00 pm. Samples were served on a tray to the judges seated in light controlled testing booths. Each tray contained two reference samples, marked "0" and "Ref," and six test samples coded with three-digit randomly selected numbers. The "0" reference contained no added fatty acid and the "Ref" references contained a high level of the fatty acid being tested. The yes-no type ballot was similar to that described by Patton et al. (1957) and Wyatt et al. (1965).

The fatty acid tested on a given day was identified on the ballot.

For butyric, hexanoic and octanoic acids the judges' task was to indicate whether or not they could detect a difference between the "0" reference and the coded butter samples. Due to the persistent soapy after-taste associated with decanoic and dodecanoic acids, duo-trio tests (Amerine et al. 1965) were used for determining the thresholds of these fatty acids. All threshold tests were replicated three times.

Mixture threshold panel tests

Interaction thresholds were determined by the same procedure as for the individual thresholds of butyric, hexanoic and octanoic acids. Mixtures of decanoic and dodecanoic acids were tested by triangle tests (Amerine et al. 1965). Instead of adding equal subthreshold amounts of each fatty acid in the mixtures as reported by Day et al. (1963), mixtures were prepared according to ratios based on individual thresholds. For example, the ratio of the individual thresholds of butyric and hexanoic acids was approximately 1:5, and butter samples were prepared with dilutions of fatty acids in this ratio. Tests were replicated at least twice.

Table 1—FFA analyses of butter and milk fat samples (average of duplicate trials).

	Concentration in butter			Concentration in milk fat				
	mg/kg			mg/kg[1]			mg/kg[2]	
Acid	I	II	III	IV	V	VI	VII	VIII
4:0	39.1	46.3	66.4	9.3	17.1	24.5	36.0	38.4
6:0	42.2	47.8	26.4	5.8	10.7	13.5	17.8	17.0
8:0	0.0	26.8	7.4	20.3	17.8	14.8	22.8	17.8
10:0	201.4	87.1	167.1	54.7	41.5	38.0	38.8	59.0
12:0	175.5	60.7	95.1	121.6	89.2	80.6	115.4	110.4
14:0	216.9	124.4	147.5	346.6	278.5	263.5	301.2	219.2
16:0	512.1	354.6	429.3	1084.0	710.2	641.1	620.2	650.8
18:0	197.9	78.5	182.3	320.1	197.2	202.9	329.2	282.8
18:1	659.5	316.0	568.4	1078.0	725.3	616.1	1050.8	1019.2
18:2	146.2	37.8	90.4	107.3	102.8	77.9	127.4	118.8
18:3	49.4	18.0	87.1	38.3	40.2	20.9	73.6	76.8

[1] Iyer et al. (1967).
[2] Bills et al. (1963).

Table 2—Average flavor thresholds of individual free fatty acids C_4-C_{12} in sweet cream butter.

FFA	Added AFT in mg/kg[1]	Total AFT in mg/kg[1,2]	Total AFT in moles ($\times 10^{-4}$) FFA/kg
C_4	11.4 ± 2.1	50.5 ± 15.8	5.7
C_6	51.5 ± 9.8	93.7 ± 29.7	8.1
C_8	454.6 ± 87.1	454.6 ± 144.2	31.5
C_{10}	161.6 ± 37.9	363.0 ± 140.9	21.1
C_{12}	127.9 ± 30.0	303.4 ± 117.8	15.1

[1] 95% confidence limit on AFT.
[2] Endogenous FFA level (Table 1, Butter Sample I) plus added FFA.

Table 3—Flavor thresholds of C_4-C_{12} free fatty acids in various media.

	Threshold concentration in ppm				
FFA	Added sweet cream butter	Total sweet cream butter	Water[1]	Neutral Edible oil[2]	Milk[1]
4:0	11.4	50.5	6.8	0.6	25
6:0	51.6	93.8	5.4	2.5	14
8:0	454.7	454.7	5.8	350.0	—
10:0	161.6	363.0	3.5	200.0	—
12:0	127.9	303.4	...	700.0	—

[1] Patton et al. (1964).
[2] Feron et al. (1961).

Preference panels

Student preference panels of 150 members were conducted for each individual FFA in butter. Students serving on the panel were not selected for taste acuity. A hedonic scale from like extremely (nine) to dislike extremely (one) was used. Each student panel member was given four coded butter samples and a hot roll. One sample was sweet cream butter and the remaining three butter samples contained varying amounts of added fatty acids.

Statistical analysis

A linear regression of correct positive responses on FFA concentration was run on each replication of the individual and mixture threshold panel data, and on the duo-trio panel data. The FFA concentration at the 50% level of positive responses was reported as the average flavor threshold (AFT). In mixture threshold interactions determinations, each component was tested separately for significance because the mixture ratios were approximations of individual thresholds.

For statistical analysis of the mixture threshold data, a transformation was made to approximate common variances for all compounds by dividing the AFT of each compound in a mixture by the AFT obtained when the compound was evaluated by itself. A pooled variance term from the individual and mixture AFT was used in the t test for interaction significance. Confidence limits of 95% were calculated for the 50% positive response threshold.

The triangle tests were evaluated by using the 5 and 1% levels from the "Significance in Triangular Test Tables" in Amerine et al. (1965). The preference test data were analyzed by analysis of variance and the least significant difference (LSD) determined at both the 5 and 1% levels.

RESULTS & DISCUSSION

RESULTS of FFA analyses of the three sweet cream butter samples used for the panel testing are presented in Table 1. Values for butteroil samples analyzed by Iyer et al. (1967), and Bills et al. (1963) are given also for comparison.

The levels of short-chain fatty acids found in butter were considerably higher than those reported for butteroil. This is probably due to the fact that intact butter samples were used rather than isolated milk fat. The short-chain fatty acids would partition more favorably into the aqueous phase, and thus would be measured more completely when intact butter is analyzed instead of isolated milk fat.

Results of the individual fatty acid threshold panels are presented in Table 2. Values are presented for both added and total FFA levels at the AFT in the butter. Butyric and hexanoic acids were generally detected by both odor and taste when added to butter samples. The results show that as chain length increased the AFT increased to a maximum for octanoic acid. Decanoic and dodecanoic acids had lower thresholds than octanoic acid, and were detected primarily by their characteristic soapy after-taste. Interpretation of the AFT values on a molar basis rather than a weight basis gave the same general threshold pattern (Table 2).

The interpretation of threshold data are difficult when the product already contains an endogenous level of the class of compounds tested. The amount of FFA present in salt or acid form in butter depends on the pH of the medium and the pKa of the acids involved. Although the Henderson-Hasselbach equation is most applicable for ideal aqueous systems, it can be used to predict approximate salt-acid ratios in complex biological systems. Therefore, butter with a pH of 6.5 would yield a 45:1 salt to acid ratio. This is probably one reason for butyric and hexanoic acids having lower thresholds in water than in milk even though milk contains endogenous FFA. Table 3 summarizes threshold data for fatty acids in various media.

The threshold values for both the added AFT and the total AFT of mixtures of FFA in butter are presented in Table 4. The t-test for interaction significance compares the AFT of each individual FFA alone with the AFT of each FFA in the mixture.

In this type of statistical evaluation, it is possible for zero one or more of the components in a mixture to show a significant flavor interaction. The calculated t-values for the mixture of butyric and octanoic acids illustrate this point. The t-values for butyric and octanoic acids at their added AFT levels were -4.481 and -4.684, respectively. These relatively large negative t-values reveal that the concentrations of both acids at their AFT in the mixture were significantly lower (at 0.01% level) than the concentration of each at its AFT alone. When total AFT's were evaluated for the same mixture, a t-value of -2.371 was obtained for octanoic acid. This showed that there was a significant flavor interaction at the 0.05% level, but not at the 0.01% level. On the other hand, the t-value for butyric acid in the same mixture was $+0.035$. This positive t-value indicates that the concentration of butyric acid in the mixture at the total AFT was higher than its concentration at its AFT alone, i.e., no flavor interaction effects were noted.

Many negative t-values were found for components of the mixtures which were not statistically significant. These t-values, although not statistically significant at either the 0.05 or the 0.01% level, indi-

Table 4—Average flavor thresholds of mixtures of free fatty acids in sweet cream butter.

FFA mixture	Added AFT[1] mg/kg	Calculated t-value	Total AFT[2] mg/kg	Calculated t-value
Butyric, Hexanoic				
Butyric	4.5 ± 2.6	−4.077[3]	50.7 ± 19.6	+0.020
Hexanoic	16.7 ± 12.1	−4.566[3]	64.5 ± 36.4	−1.271
Butyric, Octanoic				
Butyric	4.6 ± 2.1	−4.481[3]	50.9 ± 16.0	+0.035
Octanoic	171.4 ± 87.0	−4.684[3]	217.9 ± 144.0	−2.371[4]
Butyric, Decanoic				
Butyric	7.2 ± 2.1	−2.711[3]	53.5 ± 16.0	+0.027
Decanoic	140.9 ± 30.9	−0.862	228.2 ± 114.9	−1.512
Butyric, Dodecanoic				
Butyric	6.8 ± 2.6	−2.695[3]	52.6 ± 19.6	+0.168
Dodecanoic	70.0 ± 30.0	−2.781[3]	130.8 ± 117.8	−2.114[4]
Hexanoic, Octanoic				
Hexanoic	13.2 ± 12.1	−5.007[3]	61.0 ± 36.4	−1.421
Octanoic	148.2 ± 106.6	−4.542[3]	174.9 ± 176.5	−2.505[3]
Hexanoic, Decanoic				
Hexanoic	25.2 ± 12.1	−3.444[3]	73.0 ± 36.4	−0.898
Decanoic	132.8 ± 37.9	−1.095	201.8 ± 140.9	−1.650
Hexanoic, Dodecanoic				
Hexanoic	26.0 ± 12.1	−3.349[3]	73.7 ± 36.4	−0.870
Dodecanoic	71.5 ± 30.0	−2.714[3]	132.7 ± 117.8	−2.091[4]
Octanoic, Decanoic				
Octanoic	367.8 ± 106.6	−1.287	394.9 ± 176.5	−0.534
Decanoic	173.2 ± 37.9	+0.443	260.3 ± 140.9	−1.051
Octanoic, Dodecanoic				
Octanoic	335.6 ± 106.6	−1.760[4]	362.4 ± 176.5	−0.825
Dodecanoic	84.0 ± 30.0	−2.110[4]	143.4 ± 117.8	−1.959[4]

[1] AFT with 95% confidence limits.
[2] Endogenous free fatty acid level (Table 1, Butter Sample II) plus added FFA.
[3] Significant at 0.01% level ($t = -2.457$ for 30 degrees of freedom).
[4] Significant at 0.05% level ($t = -1.697$ for 30 degrees of freedom).

Table 5—Preference panels mean flavor scores of FFA concentrations in sweet cream butter.

Added mg/kg	Total[1] mg/kg	Mean score	LSD
	Butyric		
0.0	66.4	6.45	
8.2	74.6	6.44	0.01 = 0.3276
16.5	82.9	6.39	0.05 = 0.2492
33.0	99.4	5.93	
	Hexanoic		
0.0	26.4	6.24	
40.0	66.4	6.38	0.01 = 0.3368
80.0	106.4	6.06	0.05 = 0.2562
160.0	186.4	5.96	
	Octanoic		
0.0	7.4	6.44	
576.4	583.8	5.96	0.01 = 0.3721
929.5	936.9	5.19	0.05 = 0.2831
1639.0	1646.4	5.15	
	Decanoic		
0.0	167.1	6.56	
112.0	279.1	6.55	0.01 = 0.3575
225.0	392.1	6.16	0.05 = 0.2720
450.0	617.1	5.56	
	Dodecanoic		
0.0	95.1	6.62	
82.5	177.6	6.67	0.01 = 0.3239
165.0	270.1	6.53	0.05 = 0.2465
330.0	425.1	6.08	

[1] Endogenous FFA level from Table 1, Butter Sample III plus added FFA.

cate a general tendency for the fatty acids to participate in flavor interactions. Considering only added AFT data, butyric and hexanoic acids always exhibited significant flavor interactions. The same was true for the soapy-tasting dodecanoic acid containing mixtures. Octanoic acid showed significant flavor interactions for all added AFT mixtures except that of octanoic and decanoic acids. Decanoic acid did not exhibit any significant interaction in any of the mixtures.

When total AFT's were evaluated (Table 4), only octanoic acid in mixtures with butyric and hexanoic acids and decanoic acid in mixtures with butyric, hexanoic and octanoic acids showed significant flavor interactions. While butyric, hexanoic and dodecanoic acids appeared to be highly significant when considering added AFT values, only octanoic and dodecanoic acids were found to be significant in flavor interactions at the total AFT. These data emphasize the importance of consideration of endogenous levels of FFA (and other flavor compounds) present in the testing medium.

The added AFT values for the mixture of the soapy-tasting decanoic and dodecanoic acids were determined by triangular tests to be 96.9 and 76.7 ppm, respectively, and the total AFT values were 184.0 and 137.4 ppm, respectively (at the 95% confidence limit).

Caution should be used in comparing these data directly with the values given in Tables 2 and 4. Triangular test thresholds are determined by significance tables where $p = 1/3$, and thus are sample number dependent. If the 50% positive response level were used instead of the 95% significance level, AFT levels probably would be lower than those observed using triangular tests.

The results of the preference panel tests on butter containing the respective FFA added at approximate threshold and higher levels are given in Table 5. For butyric acid, the panel did not show a significant decrease in preference until a total level of 99.4 ppm was attained. This level was about twice the total AFT obtained for butyric acid by the difference panel (Table 2). However, for the remaining fatty acids studied, the total concentrations at which significant preference flavor score decreases occurred were within the confidence limits established by the individual FFA threshold determinations (Table 2).

The determination of AFT values for FFA in butter allows an estimation of the importance of each acid in butter flavor. For example, the total AFT of octanoic acid was much higher than that usually found in fresh sweet cream butter. Even though high levels of octanoic acid showed significant flavor interactions, the levels found in fresh butter would indicate that it is of only minor importance in fresh butter flavor. However, octanoic acid may be highly important in hydrolytic rancidity flavors in dairy products. This study has demonstrated interactions between pairs of compounds in the presence of endogenous levels of the same class of compounds as well as other nonacid flavor components in sweet cream butter. It is probable that all contributing flavor components interact to yield complete sweet cream butter flavor.

In summary, FFA thresholds in butter show a threshold pattern dependent on chain-length. Butyric acid exhibited the lowest total AFT for the more volatile FFA, and the AFT values increased as chain-length increased for hexanoic and octanoic acids. A soapy after-taste predominated at and above the AFT for decanoic and dodecanoic acids. Threshold values decreased from octanoic acid through dodecanoic acid as the chain-length increased. Mixture threshold results support the concept that flavor components interact at subthreshold concentrations. Endogenous FFA levels must be considered when testing products already containing FFA as part of their natural flavor. A decrease in preference was found for butters containing suprathreshold levels of added FFA.

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